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APPLICATION NUMBER: 60/136,780

FILING DATE: May 28, 1999

## PRIORITY DOCUMENT

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*L. Edelen*

L. EDELEN  
Certifying Officer

## PROVISIONAL APPLICATION COVER SHEET

is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (b)(2).

Docket Number		6013-76"USPR" FC/1d		Type a plus sign (+) inside this box*	
INVENTOR(s)/APPLICANT(s)					
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)		
QUIN	Paul		Charny, Quebec, Canada		
ASSON	Jocelyne		Ste-Foy, Quebec, Canada		
VACHON	Jean-François		Ste-Foy, Quebec, Canada		
FLISS	Ismail		Ste-Foy, Quebec, Canada		
TITLE OF THE INVENTION (280 characters max)					
INACTIVATION OF FOOD SPOILAGE AND PATHOGENIC BACTERIA BY DYNAMIC HIGH-PRESSURE HOMOGENIZATION (HPH)					
CORRESPONDENCE ADDRESS					
France Côté SWABEY OGILVY RENAULT 1981 McGill College Avenue, Suite 1600, Montréal					
STATE	Québec	ZIP CODE	H3A 2Y3	COUNTRY	Canada
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification	Number of Pages	5	<input type="checkbox"/> Small Entity Statement		
<input checked="" type="checkbox"/> Drawings	Number of Sheets	2	<input type="checkbox"/> Other (specify)		
METHOD OF PAYMENT (check one)					
<input type="checkbox"/>	A check or money order is enclosed to cover the Provisional Filing fees			PROVISONAL FILING FEE AMOUNT (\$)	\$150.00
<input checked="" type="checkbox"/>	The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number:			19-5113	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No☐ Yes, the name of the U.S. Government agency and the Government contract number are: \_\_\_\_\_

Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME

France Côté

Date

May 28, 1999

REGISTRATION NO.  
(if appropriate)

37,037

☐ Additional inventors are being named on separately numbered sheets attached hereto.

## PROVISIONAL APPLICATION FILING ONLY

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*Authors of the invention :*

Giasson Jocelyne  
Vachon Jean-François  
Fliss Ismail  
Paquin Paul

*1. Title of the invention :*

- Inactivation of food spoilage and pathogenic bacteria by dynamic high-pressure homogenization (HPH).

*2. Domain of the invention :*

- The invention is applied to food for inactivation of pathogenic bacteria. Dynamic high-pressure homogenization is used as a new alternative technology for preservation and safety products (milk, cheese, water, by-products, etc.)

*3. Commercial applications of the invention :*

- The high-pressure homogenization has the capability to kill microorganisms in milk and dairy or food products (cheese,...) and is used as a new food preservation technology. This process do not damage milk quality and allows a higher bactericidical effect than that obtained by thermal processing (pasteurization) and high hydrostatic pressure.
- The high-pressure homogenization is an alternative method for the treatment of water and the inactivation of enteric viruses such as hepatitis A, rotavirus and Norwalk virus.
- The high-pressure homogenization will be applied in inactivating bacteriophages of lactic acid bacteria.
- High-pressure homogenization could also be used as disruption process for large-scale production of intracellular high-valued products (proteins, enzymes and important care products such interferons, antibiotics and vaccines).

#### 4. Summary of the invention :

- The invention consists in using dynamic high-pressure homogenization for inactivation of food pathogens. Different pathogenic strains were used to inoculate buffer solution and milk samples at different concentrations ( $10^4$  to  $10^9$  CFU/ml). The cell suspensions were treated by high pressure homogenizer at 1 to 3 kBar with 1 to 5 recirculations (high pressure homogenizer Avestin equipment is used). The experiments were carried out at temperature ranging from  $4^\circ$  to  $55^\circ\text{C}$  and at pH ranging between 5,0 to 9,0 with different buffer solutions. The bactericidal effect was estimated by determining the residual bacterial count on plate count agar.

#### 5. Description of the invention :

- The cell suspensions are pressurized by dynamic high-pressure homogenizer using pressures ranging from 1 to 3 kBar. The disruption device used in these applications was EmulsiFlex, model C-50 from Avestin. The potential to kill bacteria by combinations of high pressure and number of recirculations obviously exists, at room temperature ; for each pressure (1, 2 and 3 kBar), the cell suspension is recirculated 1, 3 and 5. The invention is said to give important inactivation for pressure 2 kBar - 3 recirculations for Gram-positive bacteria and pressure 1 kBar - 3 recirculations for Gram-negative bacteria.
- The high pressure homogenization is carried out by a continuous process that destroy microorganisms. The resistance of microorganisms to high-pressure homogenization is variable ; the inactivation of pathogenic bacteria group, Gram-positive (*Listeria monocytogens*) and Gram-negative (*Escherichia coli*, *Salmonella enteritidis*) has been studied. These strains were resuspended in phosphate buffer at pH 7,3, or inoculation in raw milk, to a population of  $10^8$  to  $10^9$  CFU/ml. Generally, the Gram-positive bacteria are inactivated at higher pressures than Gram-negative bacteria. A total destruction of Gram-positive bacteria is obtained at 3 kBar after 3 recirculations into equipment whereas at 2 kBar pressure is sufficient to achieve a total inactivation of Gram-negative bacteria.
- Different cell populations have been treated by dynamic high-pressure homogenizers and samples were submitted to 2,5 kBar pressure, 1 recirculation at room temperature. The cells are harvested by centrifugation and resuspended in phosphate buffer to a population of  $10^4$  to  $10^9$  CFU/ml. In general, a better inactivation rate was obtained with low initial bacterial counts. For Gram-positive bacteria, a total reduction is obtained with initial counts of  $10^5$  CFU/ml whereas the same effect is obtained with initial counts of  $10^7$  CFU/ml for Gram-negative bacteria.

- The effectiveness of dynamic high-pressure homogenization is affected by cell suspension temperature. The homogenization (2.5 kBar - 1 recirculation) is carried out temperature ranging from 4° to 55°C. Higher temperature (55°C) prior high-pressure homogenization, significantly increases the bactericidal effect that has been obtained. The synergic effect between heat and high-pressure homogenization is more significant for Gram-positive bacteria probably because of the cell wall structure.
- The pathogenic cell suspensions were treated by high-pressure homogenization (2,5 kBar - 1 recirculation - room temperature) and buffer solution pH ranging was adjusted from 5,0 to 9,0. No pH effect was observed on Gram-negative bacteria. However for Gram-positive bacteria, increasing the pH from 5 to 9 is associated with increase in the susceptibility of bacteria to high-pressure homogenization.
- The effectiveness of high-pressure homogenization as an alternative method to inactive food pathogens was compared to thermal processing (pasteurization), microfiltration and hydrostatic high pressure. The dynamic high-pressure homogenization is more effective than hydrostatic high pressure (at same pressure) and than pasteurization.
- The specific design of the chamber of the high-pressure equipment is critical in the process. The chamber of the Avestin EF C-50 is different than a conventional homogenization flat head valve with stainless steel valve needle shape design. Other type of valve design can be used (ceramic flat head). The cell suspension is forced through an adjustable restricted-orifice discharge valve.
- Microorganisms are disrupted by a multiplicity of mechanisms: the sudden pressure drop, shear stresses, cavitation and impingement. The overall pressure drop and the rate at which it occurs can be responsible for the cell disruption.
- The structure of bacteria varies considerably between Gram-positive and Gram-negative organisms. It is generally accepted that the cell walls of Gram-positive bacteria are more rigid due to the thick murein layer. The Gram-negative cell walls contain thin murein layer, but they are additionally coated with a thin lipopolysaccharide layer. For these reasons, high-pressure homogenization was shown to have stronger effects on Gram-negative microorganisms.

#### 6. Principal applications of the invention :

The high-pressure homogenization is a new processing technology for :

- Extending normal shelf life of fresh food while at same time maintaining nutritional quality and ensuring safety (milk, cheese).
- Inactivating food spoilage microorganisms and food-borne diseases (alternative procedure to thermal processing and irradiation).
- Inactivating food pathogens such *Listeria monocytogens*, *Escherichia coli* and *Salmonella* in milk has been evaluated by comparaisn to pasteurization, to microfiltration and to hydrostatic high-pressure.
- Treatment of water and the inactivation of enteric viruses such hepatitis A, rotavirus and Norwalk virus and parasites.
- Eliminating lactic acid bacteria bacteriophages from cheese plant by treating milk and whey samples.
- Large-scale production of intracellular high-valued products such as nucleic acids, enzymes, proteins.

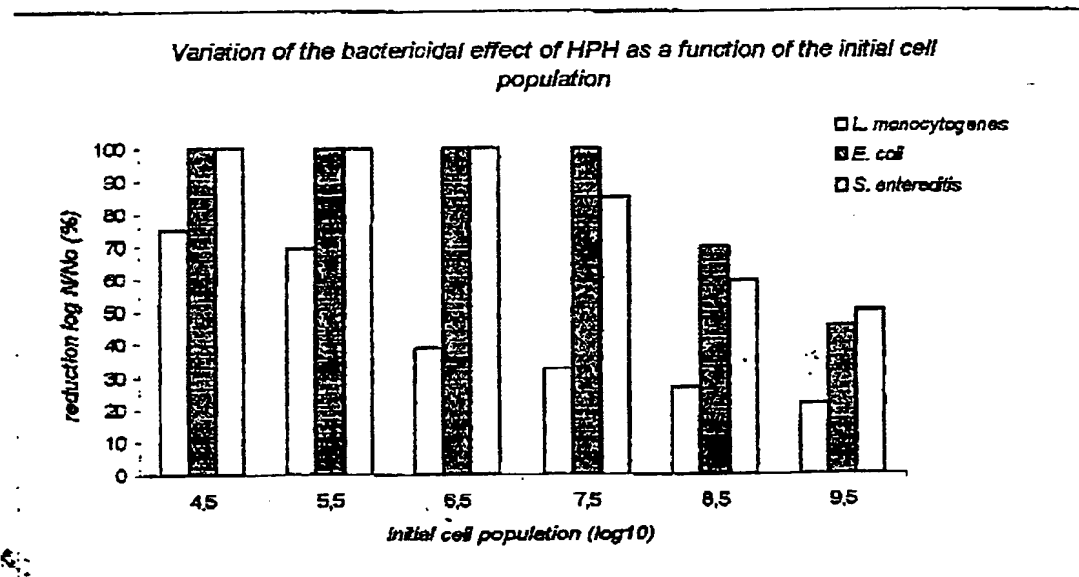
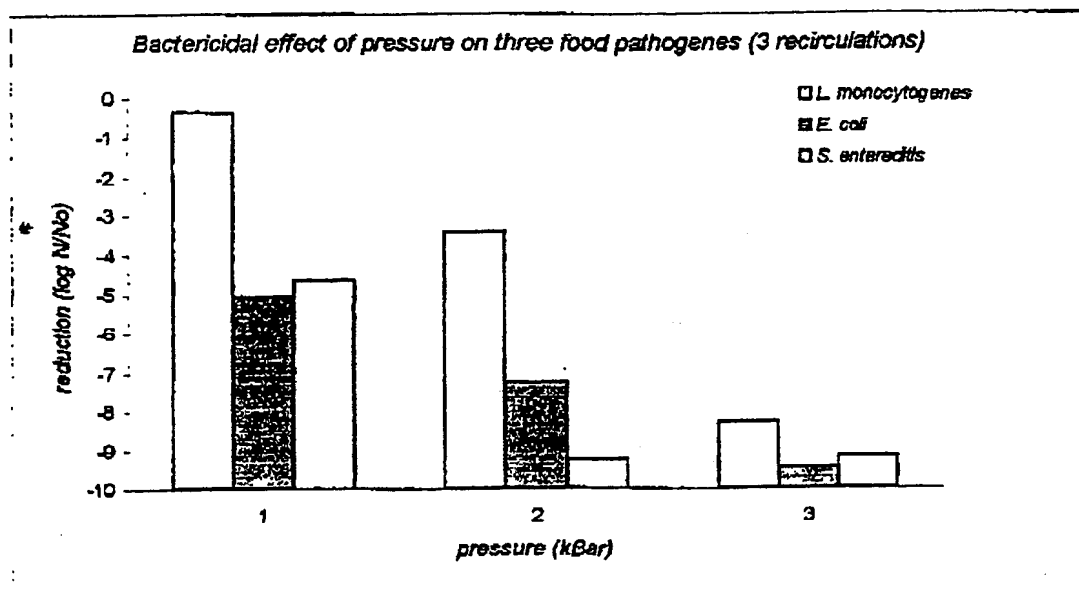
#### 7. Anterior art and literature :

- There is a need for technological advances in methods for extending the shelf life and safety of perishable foods. High hydrostatic pressure as been used for large scale production of complex and high-valued biotechnology products, intracellular products such enzymes, nucleic acids (Sauer, Robinson and Glick 1989, Popper and Knorr 1990). These process technology induces a number of changes te the morphology, biochemical reactions, genetic mechanisms, and cell membrane and wall of microorganisms. However, pressure ranging from 3 kBar to 7 kBar are necessary to kill microorganisms (Liberty, Hodzic and Aureli 1996).
- The use of high-pressure homogenization to inactivate food pathogens have never been reported. In contrast to hydrostatic high pressure treatment, the dynamic high pressure used low pressure, as about 2 kBar (Popper and Knorr 1990, Branks 1993) to achieve same bacteria inactivation results. At this pressure, food constituents are less damage. The disruption bacteria to be proportional to the number of recirculation and the operating pressure (Sauer, Robinson and Glick 1989).

- Using a Avestin Emulsiflex high-pressure homogenizer allows continuous flow operations while hydrostatic high pressure uses batch operations. A greater disruption of bacteria is possible with dynamic high-pressure, compared to other high-pressure devices.

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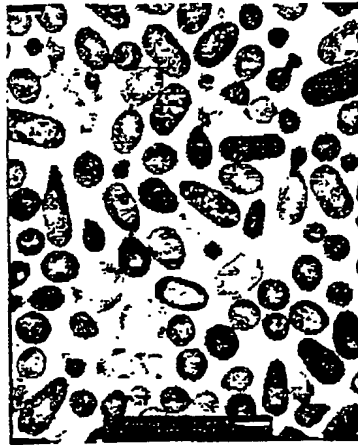
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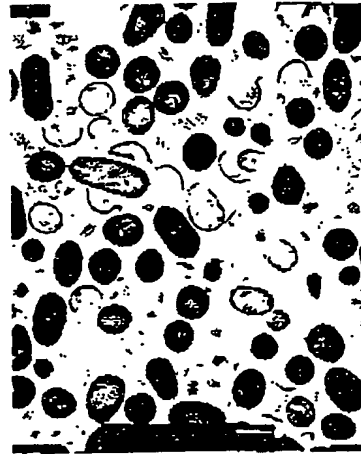
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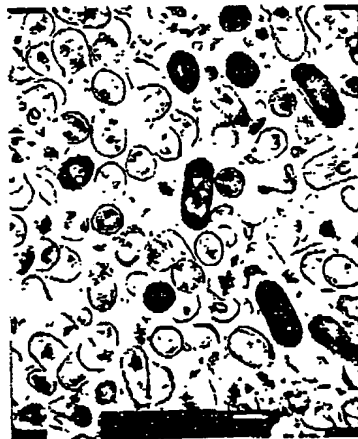
*Listeria monocytogenes*  
before and after treatment high-pressure homogenization



control



1 kBar, 1 recirculation



2 kBar, 1 recirculation



3 kBar, 1 recirculation

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